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COCCOSPORA AGRICOLA GODDARD, ITS SPECIFIC STATUS, RELATIONSHIPS, AND CELLULOLYTIC ACTIVITY

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(WITH 7 FIGURES)

OBJECTIVE

The purpose of this paper is threefold: (1) to re-establish the identity of the species *Coccospora agricola,* which has been more or less submerged and forgotten since it was described by H. N. Goddard in 1913; (2) to present certain evidence and conclusions concerning its natural phylogenetic affinities; and (3) to consider its cellulolytic activity, especially in relation to allied forms. It was through a study of cellulose-destroying fungi that the species first came to our attention.

INTRODUCTION AND REVIEW

The genus *Coccospora* Wallroth has not been subjected to any modern investigation. Its six or seven species are supposed to form sporodochia on plant substrata. It is considered to be a genus of the Tuberculariaceae.

The single species *Coccospora agricola* Goddard, with which this paper deals, is a soil-inhabiting form. On agar media it produces an effuse growth, bearing spores on short side branches in a cottony mycelium. It definitely is Moniliaceous. It has in common with the other species of *Coccospora* large globose or subglobose spores, which are 1-celled, hyaline or nearly so, and which have smooth, or slightly surface-wrinkled, unusually thick walls.

Aside from this elementary morphology there is little or no information on such fungi. Perhaps if other species of *Coccospora* were grown on agar media they too would produce the same effuse type of sporulation which is produced by *C. agricola.* Whether or not *C. agricola* and any or all of the other species of the genus are closely related phylogenetically will be determined only after further investigation on the morphology and physiology of all of the species.

The status of the genus *Coccospora* itself needs to be re-examined, especially in relation to *Sphaerosporium* Schw. The latter has nomenclatural priority and rests on a firm foundation, with the common wood-inhabiting *S. lignatile* Schw. as the type. The type species of *Coccospora, C. aurantiaca* Wallr., is of doubtful identity. It may or may not be the same species as that on which *Sphaerosporium* is based. It is not improbable that *Coccospora* will have to be referred to synonymy under *Sphaerosporium* or else discarded and the species distributed in *Sphaerosporium* and other genera.

It is evident, and will be more so after discussion further on in this paper, that nomenclatural readjustment eventually will be required for *Coccospora agricola* and many of the similar or related species of fungi. It will not be attempted here, however, for it is our conviction that the practice of name-changing in general has been taken too lightly, that it has proceeded at a rate out of all proportion to the rate of gaining new fundamental knowledge on which to base a sounder nomenclature. It is our belief that science will lose nothing if the names of such fungi as we are dealing with here are left alone for a few years until we learn more about the organisms themselves.

The species, *Coccospora agricola,* assuming that it is not buried in the literature under a still older name, was discovered by H. N. Goddard (**1913**) in garden soil in Michigan. He isolated it only once. The object of his study was to determine whether or not soil fungi were capable of fixing nitrogen. He obtained negative results for this isolate as well as for those of the several other species which he studied. He described and illustrated the species on the basis of his single isolate, but no evidence can be found that he preserved a culture or specimen. Traaen (**1914**) of Norway, in setting up his Dematiaceous genus *Humicola,* noted Goddard's *Coccospora,* but believed it to be generically distinct from his two proposed species of *Humicola* because of its thicker walled aleuriospores and lack of phialospores.

The only information on the biology of the species is that provided by Jensen (**1931a, 1931b**) in a study of the activities of the soil fungi in England and Denmark. Jensen found it to be extreme

in its intolerance of acidity and tolerance of alkalinity. He found that addition of cellulose to both acid and alkaline soils caused a multiplication of cellulolytic fungi but that totally different floras developed. The fungi developing in neutral and alkaline soil consisted of a very limited number of species, all of which were present only in very small numbers in the original soil. One of these he believed to be *Coccospora agricola,* but he did not claim certainty of identification. It proved in pure culture tests to be among the very strongest of the cellulose-decomposing fungi that were encountered. Jensen informs us (personal communication) that no culture or specimen of what he supposed to be *Coccospora agricola* was preserved. It is, however, our personal opinion that he actually did have Goddard's species.

In the same year Saccardo (**1931,** p. 633) merely presented a translation of the original description. Recently Gilman (**1945,** p. 365) excluded it from his keys and descriptions of soil fungi because of insufficient data concerning its position and occurrence.

Inquiries addressed to the curators of several herbaria and culture collections in this country and abroad have brought replies to the effect that there were no specimens or cultures on file under Goddard's binomial and that the species was unknown. It appears then that Jensen is the only worker who has identified anything with this species. It is pertinent to note also that no material has been discovered from such likely sources as cultures reported in the literature under "*Monotospora* sp.," or "*Humicola* sp."

CULTURAL CHARACTERS

One dried specimen and three cultures are here referred to *Coccospora agricola,* as follows: QM *51j,* on tentage taken in Territory of Hawaii in 1944; QM *336* (= Covert *8,* Harvard *931*), isolated at University of Pennsylvania December 1945 by Scott V. Covert from peat collected in Michigan; QM *337* (= Covert *19,* Harvard *336*), isolated at University of Pennsylvania December 1945 by Scott V. Covert from peat collected in Michigan; QM *991* (= Ajello *442,* Harvard *231*), isolated at Knoxville, Tennessee, November 1948 by Libero Ajello as a contaminant in a test tube that had been inoculated with the spleen of a rat.

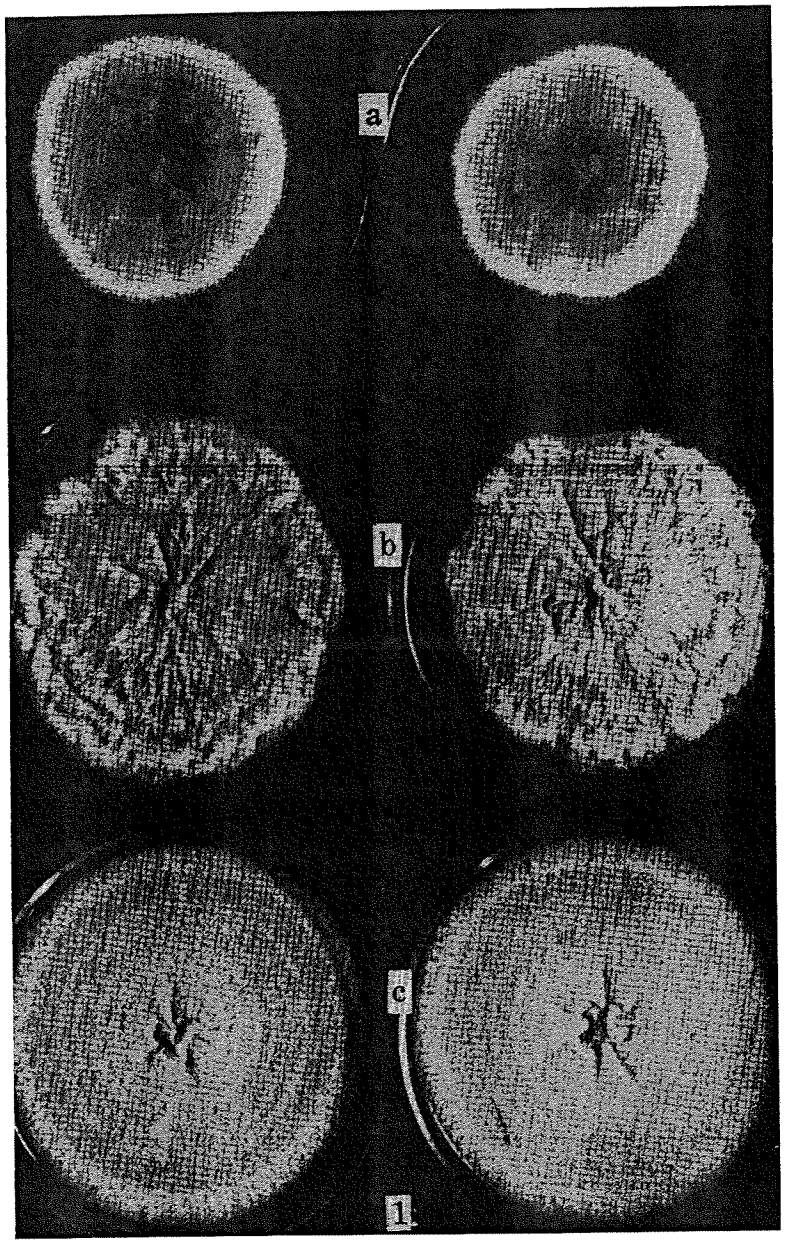

FIG. 1.

No. QM *51j* consisted of a small amount of pale ochraceous, hyphomycetous growth on a sample of deteriorated tentage. Several organisms were sporulating on the sample and several additional forms were obtained through cultural procedures. Most prominent on the sample was *Memnoniella echinata*. Some *Gliomastix convoluta* was also present. Both are strong cellulose destroyers. The *Coccospora* was closely associated with these but nothing can be said concerning any interaction. The *Coccospora* was not obtained in culture. Nor was any special effort made to obtain it, since critical surface examination of the sample was not made at the time of culturing. Spores mounted in KOH solution were globose or nearly so, golden yellow with the color apparently in the walls, variable in diameter, 13–22 μ, the content scarcely granular, the wall 3–3.5 μ thick and entirely smooth. No tendency for the wall-layers to become separated in the mounting fluid was noticed. It is with some hesitation that this is referred to *C. agricola*.

Gross characters of cultures. Cultures on the usual agar media grow rapidly and sporulate quickly and copiously. The mycelium is white or whitish at first, but with sporulation the colony becomes uniformly buff. In FIG. 1 is shown duplicate plates of the three cultures, QM *336* (FIG. 1A), QM *337* (FIG. 1B), and QM *991* (FIG. 1C), grown in parallel series on potato dextrose agar as indicated in the legend. These were started by transferring bits of mycelial growth from a similar agar culture. The colonies become "Tilleul-Buff" (Ridgeway) by the third or fourth day. QM *337* (FIG. 1B) and *991* (FIG. 1C) remain that color permanently. In QM *336* (FIG. 1A), however, there soon appear numerous sterile unbranched hair-like structures (FIG. 5), at first in concentric rings in the older central area (FIG. 1A), gradually becoming more numerous until at about the end of a month they impart a medium gray *Alternaria*-like aspect to the entire colony. Such hairs also appear occasionally in faint concentric rings in old cul-

FIG. 1. *Coccospora agricola*. A, QM *336;* B, QM *337;* C, QM *991*. Grown in parallel series under room conditions on potato dextrose agar (20 g. dext., 20 g. agar, water from 400 g. potatoes, made up to 1 liter), 35 cc. of the medium per dish. × 0.8. (Photo by Frank White, Dept. Biology, Harvard Univ.)

FIG. 4.—*Coccospora agricola*, n. sp.: *a*, habit sketch; *b*, branched hypha, ×80; *c*, hyphae bearing conidia on simple side branches, ×180; *d*, clustered conidiophores, ×380; *e*, simple conidiophores bearing conidia, ×380; *f*, conidia (chlamydospores), ×380.

FIGS. 2–7.

tures of QM *337* but never in such prominence as to impart their dark coloration to the colony. They have never been observed in *991*. Certain other differences are apparent in FIG. 1. QM *337* is characterized by sectors which produce an overgrowth and others in which this is absent. Heavy sporulation is present on the mycelium comprising the overgrowth as well as on that underneath. Where the overgrowth is absent the surface of the colony is much the same as that of QM *991* (FIG. 1C). The cultures have not been compared in an extensive range of media. During the period of approximately five years that two of them have been carried in stock, however, they have been grown in plate culture and photographed several times. These records present evidence of extreme plasticity, even on more or less the same medium. One photo of *336* shows no visible evidence of the hair-like growths after two months and one of *337* shows the growth developed in deep conspicuous concentric rings.

Microscopic characters. Aside from the presence or absence of the dark hairs, no differences have been noted in the three cultures. Spores from the petri plates shown in FIG. 1, mounted in water, gave the following measurements:

Culture	Range	Average of 70
QM *336*	10–17μ	13.9μ
QM *337*	11–21μ	15.1μ
QM *991*	10–18μ	14.9μ

Under a good dissecting microscope they may be seen individually as they are borne rather copiously, singly, or more often in small

FIGS. 2–7. *Coccospora agricola*. 2. Goddard's original drawings reproduced from the Botanical Gazette. 3. Conidiophores with conidia attached (compare with Goddard's *d*), from culture grown 10 days under room conditions on Czapek's agar medium, mounted in 7 percent KOH solution and stained with phloxine, × 1000. 4. Conidia from same culture, mounted in 7 percent KOH, unstained. 5. Basal portion of one of the sterile dark hair-like filaments which appears among the sporulating hyphae, from a 30-day-old culture on commercial Bacto-Difco potato dextrose agar (2 per cent dext.), mounted in lacto-phenol, unstained, × 2000. 6. Four spores, perhaps slightly immature, borne on short side branches of a hypha, from 7-day-old culture on P.D.A. as above, mounted directly in aqueous phloxine, × 2000. 7. Spores showing loosened outer layer of wall, from culture grown 14 days on Czapek's agar, mounted in 7 percent KOH to which a drop of aqueous phloxine was added, × 1000. (Photos 3–5 from culture QM *336;* 6–7 from QM *337;* 3, 4, and 7 by the junior author; 2 by Frank White, and 5, 6 by Paul Brown, both of Harvard University.)

clusters, on the rather loosely arranged hyphae. Thus viewed, they appear globose, subhyaline, smooth, glistening. In microscope preparations they are hyaline, thick walled—up to 3.5 μ, with the walls perhaps slightly reticulate (FIGS. 3 & 4). In either water or KOH preparations, but especially in the latter, what appears to be an outer membrane commonly separates from the main wall (FIG. 7), ruptures, and may tend to partially disappear.

Since Goddard did not preserve any material, identification of the current cultures can be made only by comparison with his original descriptions and illustrations. His illustrations are therefore reproduced in FIG. 2. For convenience the original description is quoted here:

"*Coccospora agricola,* n. sp.—*Mycelium orbicular,* at first white with a tufted center and radiate border, becoming slightly zoned with concentric grooves and ridges, turning pinkish brown especially inside, finally forming a powdery pinkish brown surface with age; reverse side weakly orange. *Hyphae* very little branched, septate, 4–6 μ broad, hyaline. *Conidiophores* little differentiated, consisting of short side branches, each bearing a single spore at the end, or sometimes forming racemose clusters; side branches 12–30 μ long, generally septate. *Conidia* (chlamydospores) large, thick-walled, mostly globular, very persistent, not being set free in water, 16–25 μ in diameter; membrane hyaline, 2–3 μ thick; contents highly granular and faintly brownish—fig. 4.

"The large, thick-walled, persistent chlamydospores, and the simple method of fructification, were the most strikingly characteristic features of this fungus."

COCCOSPORA AGRICOLA AND THE GENUS HUMICOLA

A consideration of all characters, both morphological and physiological, convince us that *Coccospora agricola* has extremely close natural affinities with the two species upon which Traaen established his genus *Humicola,* i.e., *H. fuscoatra* Traaen (type) and *H. grisea* Traaen. All three species occur naturally as soil fungi. With the exception of *C. agricola,* QM *51j,* none of them have ever been observed in any recognizable state on any substratum under field conditions. Many cultures of *H. fuscoatra* have been obtained from deteriorated cotton fabrics, perhaps many or most of the samples having had soil contact, but the organism does not sporulate there so that it can be studied directly, in contrast, for example, with the habit of *Memnoniella echinata.* They all grow relatively rapidly and sporulate readily at room temperatures on the common

agar culture media, including filter paper and cotton fabric on a mineral salts medium. They rank among the strongest cellulose-destroyers known. Jensen's finding of a high pH preference for *Coccospora agricola* corresponds with similar findings in our own laboratory for *Humicola fuscoatra.* All three species have globose spores borne singly on short side branches. The walls are relatively thick and have a "double contour" with the outer wall, under certain conditions, tending to separate from the inner. The two species of *Humicola* are gray or dark as sporulation advances whereas *Coccospora agricola* is pale buff. The spores of *Humicola fuscoatra* are smaller than those of *Coccospora agricola,* have walls that are less thickened, and are colored in mass although when viewed singly they are subhyaline. Those of *Humicola grisea* are about the same size as those of *Coccospora agricola* but are deeply colored.

A fourth species which we would consider here is *Monotospora lanuginosa* (Griff. & Maubl.) Mason. It is similar to *Humicola fuscoatra* but has smaller spores, is thermophilic, and is not cellulolytic. It occurs in compost piles.

We plan to treat the remaining three of these four closely related species in future papers. For the present we prefer to build around the genus *Humicola.* We find it difficult to accept the thesis that *Humicola grisea* is synonymous with *Monotospora toruloides* Corda, which would make *Humicola* Traaen a synonym of *Monotospora* Corda.

CELLULOLYTIC ACTIVITY

The form with which we are mainly concerned here, *Coccospora agricola,* had been shown to be strongly cellulolytic by Covert (Ph.D. thesis, University of Pennsylvania) from whom we obtained two of our cultures. The similar findings of Jensen are also recalled at this point. During the past few years *Humicola fuscoatra,* which is the form commonly encountered by those working on fabric decay, has come to be generally known as a strongly cellulolytic species. To our knowledge *H. grisea* has not previously been tested. Reese (Ph.D. thesis, Pennsylvania State College) had found *Monotospora lanuginosa* to be non-cellulolytic. So while there was at hand considerable cumulative information, it seemed

TABLE 1

CELLULOLYTIC ACTIVITY OF *Humicola*-LIKE ORGANISMS ON 12 OZ. GREY COTTON DUCK

Culture number	Percent loss in tensile strength	Final pH of liquid	Cx activity mg./ml./hr.	Growth index	Appearance of growth	
					In test	On 0.5 per cent P.D.A. inoculum tube
Coccospora agricola QM *336*	94	6.8	.54	4	Grey, covering strip, heavier at surface of liquid	Cream colored
QM *337*	100	7.0	.40	3	White, covering most of strip, heavier at surface of liquid	Cream colored
QM *991*	100	6.8	.30	2	Heavy greyish white, at surface of liquid only	Cream colored
Humicola fuscoatra QM *34e*	100	6.9	.15	3	White over dark grey, less floccose than above	Grey appressed
QM *71c*	66	6.9	.37	4	Pinkish over light grey	Pinkish tan
QM *73d*	100	7.0	.12	4	Heavy, grey over black, heavier at liquid surface	Grey over black
QM *73e*	100	6.7	.19	4	As QM *73d*	Grey over black
QM *130e*	100	6.9	.19	3	As QM *73d* but less growth	White over grey
QM *133a*	100	7.0	.11	4	As QM *73d*	White over black
QM *136c*	100	6.7	.11	4	As QM *73d*	Grey over black
QM *154e*	100	6.8	.07	4	Pinkish over black	Pink over black
QM *161*	100	6.7	.15	4	White over black	White over black
QM *162*	100	6.7	.15	4	White over black	White over black
QM *468*	100	6.7	.12	4	White over black	White over black
QM *469*	82	6.6	.07	4	Deep yellow over brownish	Deep yellow over brownish
QM *470*	100	7.0	.06	4	Deep grey over black	Grey over black
QM *473*	100	6.7	.22	3	Spotty deep grey over black, heavier at liquid surface	Grey over black
QM *997*	41	6.5	.03	0	No growth on strip, mycelium in liquid	Grey, moist appressed
QM *999*	100	6.6	.12	4	Deep grey over black, strip yellow green	White over grey
QM *1000*	100	6.9	.22	4	Deep grey over black, not floccose	Grey over black
QM *1001*	100	6.7	.29	4	White, floccose over dark grey	White over black

TABLE 1—*Continued*

Culture number	Percent loss in tensile strength	Final pH of liquid	Cx activity mg./ml./hr.	Growth index	Appearance of growth	
					In test	On 0.5 percent P.D.A. inoculum tube
Humicola fuscoatra QM *1002*	100	6.8	.16	4	Grey over black	White over black
QM *1003*	100	6.7	.40	4	Grey over black	Grey over black
JQMD *1017*	100			4	Grey over black	Grey over black
JQMD *1021*	100			4	White over grey	White over black
JQMD *1026*	100	6.7	.50	4	Deep pink-peach over yellow-brown	Pink over pinkish brown
JQMD *1031*	77	6.7	.04	4	Small amount, white over appressed black	Grey over black
JQMD *1045*	100	6.7	.23	4	Grey over black	Grey over black
JQMD *1052*	100	6.5	.20	4	As JQMD *1045*, more appressed	Grey over black
Humicola grisea QM *228*	80	5.7	.19	3	Heavy dark grey over most of fabric; black at liquid surface	Black
*QM *228*	88			3	As at 30° C.	Black
QM *542*	100	6.6	.38	4	Heavy, grey over black, heavier at liquid surface	Grey over black
QM *992*	100	6.5	.62	4	As QM *542*	Grey over black
QM *993*	100	6.7	.32	4	As QM *542*, darker	Dark grey over black
QM *994*	100	6.7	.24	4	As QM *542*, plus slight greenish grey	Dark grey over black
QM *995*	100	6.7	.24	4	As QM *994*	Dark grey over black
QM *996*	100	6.7	.05	4	As QM *994*	Dark grey over black
Monotospora lanuginosa QM *225*	2	6.8	.02	0	No growth	
*QM *225*	3	6.8	.00	1	Mycelium at liquid surface	Tan over grey
QM *226*	3	7.3	.00	1	Small scattered grey growth darker at liquid surface	
*QM *226*	2	7.1	.00	2	As at 30° C. but heavier	Dark grey over black
QM *227*	3	7.0	.02	0	No growth	
*QM *227*	2	7.1	.00	1	Dark mycelium at liquid surface and scattered over cloth	Dark grey over black

* Incubated at 40° C.

highly desirable to run a final set of tests on most of the cultures that had come to hand.

The activity of each isolate was tested by growing on 3 × 1 inch strips of 12 oz. grey cotton duck, the decline in tensile strength being taken as the measure of cellulolytic activity. Ten replicates were used for each culture. The strips were placed 1 each in test tubes of 18 × 150 mm. size, in which the lower half of the strip was submerged in 9 ml. of a liquid medium. The tubes were plugged with non-absorbent cotton and autoclaved at 121° C. for 15 minutes. The liquid medium was composed as follows: 1 percent yeast extract, 10 ml.; 10 percent NH_4NO_3, 10 ml.; 10 percent $MgSO_4$, 3 ml.; M/1 KH_2PO_4 buffer pH 4.5, 10 ml.; dist. H_2O, 960 ml. The pH was brought to 6.4 by the addition of 4.5 ml. of N/1 NaOH. Distilled water was added to bring the total volume to 1000 ml.

For inoculum the cultures to be tested were grown on slants of 0.5 percent potato dextrose agar under room conditions for about one month. Before using, the spore surface of each slant was wet out with 2 to 3 drops of 0.5 percent solution of Aerosol O T, then 20 ml. of sterile distilled water was added to each tube and the spores brought into suspension by agitation with the tip of the pipette. A 1 ml. spore suspension was placed by means of the pipette on each cloth strip. Two sets each of QM *225, 226, 227* and *228* were inoculated, one to be incubated at 30° C. and the other at 40° C. All others were incubated at 30° C. only. Each tube was examined for the amount and type of growth and a rough numerical value given each to indicate the relative amount present 13 days after inoculation. The strips were harvested by removing them from the liquid, washing in 50 percent ethyl alcohol for 30 minutes, rinsing in tap water, drying at room temperature, conditioning at 21° C. and 65 percent relative humidity, and breaking on the Scott Tester. Measurements of pH and tests for Cx activity were made on the liquid from each representative culture. The Cx activity is reported as the amount of reducing sugar (as glucose) produced by a filtrate acting on carboxymethylcellulose (CMC 50T) in one hour at 50° C. Activities of 0.05 mg./ml./hr. or less were considered doubtful or negative. Cx is the polysaccharase hydrolyzing the 1, 4 glucosidic linkages of the cellulose chain.

It will be noted that the various isolates tested, except those of *Monotospora lanuginosa* (inactive at both 30° C. and 40° C.), proved to be uniformly highly active in the degradation of the cotton duck, and that with two exceptions (*Humicola fuscoatra,* QM *997* and JQMD *1031*) they showed Cx activity. It is evident from the data in the two right-hand columns of the table that great variation in color of colony is exhibited among the various strains within a species.

SUMMARY

Three living cultures are identified as *Coccospora agricola,* a species described by H. N. Goddard in 1913, but which has remained an unknown entity since that time. Goddard's species is considered to be re-established. It is a soil-inhabiting species, strongly cellulolytic, and marked especially by its large, globose, hyaline spores whose walls are unusually thick. Its cultural characters, morphology, strong cellulolytic activity and soil habitat are strongly suggestive of the genus *Humicola* Traaen, which contains just two species, *H. fuscoatra* (type) and *H. grisea.* Nomenclatural readjustment is deferred pending a thorough study of a wide range of similar or related fungi.

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